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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Chatterjee
Serial No.: 10/076,033
For: A PROCESS FOR THE ISOLATION OF A MAJOR HARMFUL
OXIDANT FROM CIGARETTE SMOKE
Filed: February 13, 2002
Examiner: Walls, Dione A.
Customer No.: 27623
Art Unit: 1731
Confirmation No.: 7393

Attorney Docket: 3030.003USU

**NON-FEE AMENDMENT
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450**

Dear Sir:

ELECTION OF INVENTION SPECIES TRANSMITTAL FORM

Transmitted herewith is an Election of Invention Species in the above-identified application. In accordance with the Office Action Summary dated August 21, 2003, the applicants wish to elect Species I (claims 1, 3-4, 6-19 and 41) for prosecution in this application.

Petition for extension of time pursuant to 37 C.F.R. §§ 1.136 and 1.137 is hereby made if, and to the extent, required. The fee for this extension of time is calculated to be \$_____ to extend the time for filing this response until _____.

The fee for any change in number of claims has been calculated as shown below.

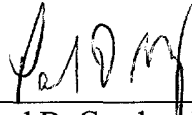
CLAIMS AS AMENDED						
	Claims Remaining After Amendment		Highest Number Previously Paid	Present Extra	Rate	
Total Claims	18	Minus	39	0	x \$18.00	\$0
Independent Claims	2	Minus	5	0	x \$84.00	\$0
MULTIPLE DEPENDENT CLAIM FEE				x \$280.00 = \$		
TOTAL FEE FOR CLAIM CHANGES				PAID		

The total fee for this amendment, including claim changes and any extension of time is calculated to be \$ 0.00.

 A check in the amount of \$ 0.00 is attached.

XXX The Commissioner is hereby authorized to charge any additional fees under 37 C.F.R. §§1.16 and 1.17 which may be required with this communication or during the entire pendency of the application, or credit any overpayment, to **Deposit Account No. 01-0467**. A duplicate copy of this Form is enclosed.

September 19, 2003
Date



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CERTIFICATE OF MAILING

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE U.S. POSTAL SERVICE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO: NON-FEE AMENDMENT, COMMISSIONER FOR PATENTS, P.O. BOX 1450, ALEXANDRIA, VA 22313-1450, ON SEPTEMBER 19, 2003.

Kenroy A. Browne
NAME



SIGNATURE

09/19/03
DATE



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

RECEIVED
SEP 25 2003
TC 1700

Applicant(s): Chatterjee
Serial No.: 10/076,033
For: A PROCESS FOR THE ISOLATION OF A MAJOR HARMFUL
OXIDANT FROM CIGARETTE SMOKE
Filed: February 13, 2002
Examiner: Walls, Dione A.
Customer No.: 27623
Art Unit: 1731
Confirmation No.: 7393

Attorney Docket: 3030.003USU

**NON-FEE AMENDMENT
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450**

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ELECTION OF INVENTION SPECIES TRANSMITTAL FORM

Transmitted herewith is an Election of Invention Species in the above-identified application. In accordance with the Office Action Summary dated August 21, 2003, the applicants wish to elect Species I (claims 1, 3-4, 6-19 and 41) for prosecution in this application.

Petition for extension of time pursuant to 37 C.F.R. §§ 1.136 and 1.137 is hereby made if, and to the extent, required. The fee for this extension of time is calculated to be \$ _____ to extend the time for filing this response until _____.

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TOTAL FEE FOR CLAIM CHANGES				PAID		

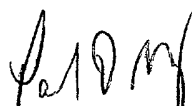
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September 19, 2003

Date



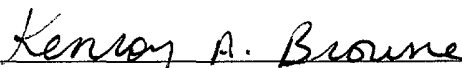
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Kenroy A. Browne

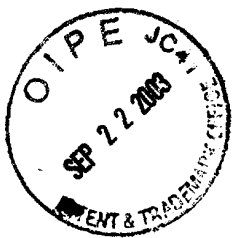
NAME



SIGNATURE

09/19/03

DATE



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

RECEIVED
SEP 25 2003
TC 1700

Applicant: Indu Bhusan Chatterjee
Serial No.: 10/076,033
For: A Process for the Isolation of a Major Harmful Oxidant from Cigarette Smoke
Filed: February 13, 2002
Examiner: Walls, Dionne A.
Art Unit: 1731

Attorney Docket No.: 3030.003USU

USPTO Customer Number: 27623

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO ELECTION/RESTRICTION

Dear Sir:

In response to the Office Action of August 21, 2003, please amend the above-identified application as follows:

Amendments to the Claims are reflected in the listing of claims, which begins on page 2 of this paper.

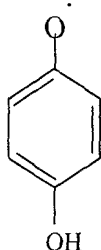
Remarks/Arguments begin on page 14 of this paper.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (original): A process for the isolation of p-benzosemiquinone of formula 1



(Formula I)

a major harmful oxidant from cigarette smoke responsible for the oxidative damage of proteins and DNA, the said process comprising the steps of

- (a) collecting tar or cs (cigarette smoke) solution from lighted conventional filtered tipped cigarettes,
- (b) collecting tar by lighting conventional filter-tipped cigarettes having a tar content of 20 -30 mg per cigarette in a glass flask dipped in a mixture of ice and salt and allowing the tar to condense and settle at the bottom of the flask,
- (c) keeping the above said flask at room temperature and extracting the said tar with 30-60 mM potassium phosphate buffer at a pH ranging between 7.4 to 7.8, filtering the above solution through 0.45 µm Millipore filter and adjusting the pH of the filtrate ranging between 7.4 to 7.6 by adding NaOH solution to obtain the desired tar solution,
- (d) extracting the above said tar solution thrice with equal volume of methylene chloride, discarding the lower methylene chloride layer and collecting the upper yellow coloured aqueous layer termed as aqueous extract of cigarette smoke.
- (e) extracting the above said aqueous extract of cigarette smoke twice with equal volume of water saturated n-butanol, lyophilizing the pooled yellow butanol extract in a

- lyophilizer at a temperature ranging between -50°C to -60°C under vacuum followed by extraction of the lyophilized material twice with HPLC grade acetone and drying the acetone solution under vacuum and dissolving the said acetone extract with HPLC grade methanol,
- (f) subjecting the above said methanol solution to band TLC using non-fluorescent silica plates, developing the said silica plates using a mixture of toluene and ethyl acetate in a ratio of 80:20, taking out the said plate and drying it at about $25-30^{\circ}\text{C}$ using a drier, cutting small strips containing the developed material from both sides of the plates and keeping them in an iodine chamber for the location of the band corresponding to R_f 0.26, scraping the band and extracting the band material with HPLC grade acetone followed by collection of the acetone layer and drying it under vacuum,
 - (g) dissolving the above said acetone extract which appeared as pale yellow needles by adding equal volume of milli Q water, extracting the resultant aqueous solution with equal volume of HPLC grade water saturated n-butanol followed by drying upper n-butanol layer in small glass tubes under vacuum to obtain the major cigarette smoke (cs) oxidant with a purity of 98-99% and yield of about 18-22 μg per cigarette,
 - (h) purifying the above said cs oxidant as obtained in step (g) by dissolving it in a mobile solvent comprising a mixture of methylene chloride and methanol in a ratio of 90:10 (v/v) and injecting it in a HPLC instrument with a normal phase 25 cm silica column using a uv detector at 294 nm at a flow rate of 0.5 ml/min, at a temperature of about 25°C and at a pressure of about 29 kgf/cm² followed by collecting the effluent which appears as a single peak at a retention time of 8.808 min with a purity of 100 % and yield of 8.4% of the total cs oxidant present in the parent tar solution.

Claim 2 (withdrawn): A process for the isolation of p-benzosemiquinone of formula 1, a major harmful oxidant from cigarette smoke responsible for the oxidative damage of proteins and DNA, the said process further comprising

- (a) passing the whole cigarette smoke collected from conventional filter tipped cigarette having a tar content of 20 -30 mg per cigarette into 30-60 mM potassium buffer at pH 7.4 -7.8, filtering the above solution through 0.45 μm Millipore filter, adjusting the

- pH of the filtrate ranging between 7.4 to 7.6 by adding NaOH solution to obtain the desired cigarette smoke solution (cs solution);
- (b) extracting the above said cs solution thrice with equal volume of methylene chloride, discarding the lower methylene chloride layer and collecting the upper yellow coloured aqueous layer termed as aqueous extract of cigarette smoke;
 - (c) extracting the above said aqueous layer of cigarette smoke twice with equal volume of water saturated n-butanol, lyophilizing the pooled yellow butanol extract in a Lyolab lyophilizer at a temperature ranging between -50°C to -60°C under vacuum followed by extraction of the lyophilized material twice with HPLC grade acetone and drying the acetone solution under vacuum and dissolving the said acetone extract with HPLC grade methanol;
 - (d) subjecting the above said methanol solution to band TLC using non-fluorescent silica plates, developing the said silica plates using a mixture of toluene and ethyl acetate in a ratio of 80:20, taking out the plate and drying at about 25°C to 30°C using a drier, cutting small strips containing the developed material from both sides of the plates and keeping them in an iodine chamber for the location of the band corresponding to R_f 0.26, scraping the band and extracting the band material with HPLC grade acetone followed by collection of the acetone layer and drying it under vacuum;
 - (e) dissolving the above said acetone extract which appeared as pale yellow needles by adding equal volume of milli Q water, extracting the aqueous solution with equal volume of HPLC grade water saturated n-butanol followed by drying the upper n-butanol layer in small glass tubes under vacuum to obtain the major cs oxidant with a purity of 98-99% and yield of 18-22 μg per cigarette; and
 - (f) purifying the above said cs oxidant as obtained in step e by dissolving it in a mobile solvent comprising a mixture of methylene chloride and methanol in a ratio of 90:10(v/v) and injecting it in a HPLC instrument with a normal phase 25 cm silica column using a uv detector at 294 nm at a flow rate of 0.5 ml/min, at a temperature of about 25°C , at a pressure of about 29 kgf/cm² and collecting the effluent which appears as a single peak at a retention time of 8.808 min with a purity of 100 % and

yield of 8.4% of the total cs oxidant present in the parent cs solution.

Claim 3 (previously presented): A process as claimed in claim 1, wherein said isolated pure cigarette smoke (cs) oxidant has the following properties:

- (a) when crystallized from acetone solution appears as small needle shaped faint yellow coloured crystals having pungent smell, similar to that of rancid butterfat,
- (b) UV absorption maxima in methanol solution are at 293.4 nm and 223.0 nm and in aqueous solution are in 288nm and 221nm, respectively,
- (c) on excitation at 293 nm in methanol solution the observed emission maxima are at 329.6 nm and 651.4 nm and on excitation at 224 nm, the observed emission maxima are at 329.6 nm and 652.6 nm, respectively,
- (d) when excitation scanning is monitored keeping the emission at 330 nm, the observed excitation maxima are at 228.2 nm and 293.8 nm and when the emission is kept at 651 nm and excitation scanning is monitored, the observed excitation maxima are at 229.2 nm and 294.8 nm, respectively,
- (e) highly soluble in methanol, ethanol, acetone, n-butanol, fairly soluble in water, sparingly soluble in methylene chloride, di-ethyl ether, chloroform and insoluble in benzene and petroleum ether,
- (f) the compound loses its oxidizing potency in acidic pH ranging between 4 to 5 and on keeping the solution at alkaline pH ranging between 9 to 10, the compound gradually turns brown, at pH 10 and above there is instantaneous darkening with loss of both activity and aromaticity as evidenced by UV spectroscopy,
- (g) the half-life of the oxidant, when stored in the solid state at a temperature ranging between 25 °C to 30 °C under darkness is about 48 hours as determined by its oxidative potency, but in solution of 50 mM potassium phosphate buffer, pH 7.4 at 25°C to 30°C the half life is about 1 hour 30 min,
- (h) reduces ferricytochrome c and ferric chloride,
- (i) oxidizes ascorbic acid, proteins and DNA, and
- (j) the melting point is 162°C.

Claim 4 (original): A process for the quantitative determination of p-benzosemiquinone of

formula 1, a major harmful oxidant isolated from cigarette smoke responsible for the oxidative damage of proteins and DNA, the said process comprising the steps of

- (a) collecting tar or cs (cigarette smoke) solution from lighted conventional filtered tipped cigarettes,
- (b) collecting tar by lighting conventional filter-tipped cigarettes having a tar content of 20 -30 mg per cigarette in a glass flask dipped in a mixture of ice and salt and allowing the tar to condense and settle at the bottom of the flask,
- (c) keeping the above said flask at room temperature and extracting the said tar with 30-60 mM potassium phosphate buffer at a pH ranging between 7.4 to 7.8, filtering the above solution through 0.45 μ m Millipore filter and adjusting the pH of the filtrate ranging between 7.4 to 7.6 by adding NaOH solution to obtain the desired tar solution,
- (d) extracting the above said tar solution thrice with equal volume of methylene chloride, discarding the lower methylene chloride layer and collecting the upper yellow coloured aqueous layer termed as aqueous extract of cigarette smoke,
- (e) extracting the above said aqueous extract of cigarette smoke twice with equal volume of water saturated n-butanol, lyophilizing the pooled yellow butanol extract in a lyophilizer at a temperature ranging between -50°C to -60°C under vacuum followed by extraction of the lyophilized material twice with HPLC grade acetone and drying the acetone solution under vacuum and dissolving the said acetone extract with HPLC grade methanol,
- (f) subjecting the above said methanol solution to band TLC using non-fluorescent silica plates, developing the said silica plates using a mixture of toluene and ethyl acetate in a ratio of 80:20, taking out the said plate and drying it at about $25-30^{\circ}\text{C}$ using a drier followed by cutting small strips containing the developed material from both sides of the plates and keeping them in an iodine chamber for the location of the band corresponding to R_f 0.26, scraping the band and extracting the band material with HPLC grade acetone followed by collection of the acetone layer and drying it under vacuum,
- (g) dissolving the above said acetone extract which appeared as pale yellow needles by adding equal volume of milli Q water, extracting the resultant aqueous solution with equal volume of HPLC grade water saturated n-butanol followed by drying upper n-

- butanol layer in small glass tubes under vacuum to obtain the major cigarette smoke (cs) oxidant with a purity of 98-99% and yield of about 18-22 μg per cigarette, and
- (h) purifying the above said cs oxidant as obtained in step (g) by dissolving it in a mobile solvent comprising a mixture of methylene chloride and methanol in a ratio of 90:10 (v/v) and injecting it in a HPLC instrument with a normal phase 25 cm silica column using a uv detector at 294 nm at a flow rate of 0.5 ml/min, at a temperature of about 25⁰C and at a pressure of about 29 kgf/cm² followed by collecting the effluent which appears as a single peak at a retention time of 8.808 min with a purity of 100 % and yield of 8.4% of the total cs oxidant present in the parent tar solution.

Claim 5 (withdrawn): A process for the quantitative determination of p-benzosemiquinone of formula 1, a major harmful oxidant isolated from cigarette smoke responsible for the oxidative damage of proteins and DNA, the said process further comprising

- (a) passing the whole cigarette smoke collected from conventional filter tipped cigarette having a tar content of 20 -30 mg per cigarette into 30-60 mM potassium buffer at pH 7.4 -7.8, filtering the above solution through 0.45 μm Millipore filter, adjusting the pH of the filtrate ranging between 7.4 to 7.6 by adding NaOH solution to obtain the desired cigarette smoke solution (cs solution),
- (b) extracting the above said cs solution thrice with equal volume of methylene chloride, discarding the lower methylene chloride layer and collecting the upper yellow colored aqueous layer termed as aqueous extract of cigarette smoke,
- (c) extracting the above said aqueous layer of cigarette smoke twice with equal volume of water saturated n-butanol, lyophilizing the pooled yellow butanol extract in a Lyolab lyophilizer at a temperature ranging between -50 ⁰C to -60⁰C under vacuum followed by extraction of the lyophilized material twice with HPLC grade acetone and drying the acetone solution under vacuum and dissolving the said acetone extract with HPLC grade methanol,
- (d) subjecting the above said methanol solution to band TLC using non-fluorescent silica plates, developing the said silica plates using a mixture of toluene and ethyl acetate in a ratio of 80:20, taking out the plate and drying at about 25 ⁰C to 30 ⁰C using a drier, cutting small strips containing the developed material from both sides of the

- plates and keeping them in an iodine chamber for the location of the band corresponding to Rf 0.26 , scraping the band and extracting the band material with HPLC grade acetone followed by collection of the acetone layer and drying it under vacuum,
- (e) dissolving the above said acetone extract which appeared as pale yellow needles by adding equal volume of milli Q water , extracting the aqueous solution with equal volume of HPLC grade water saturated n-butanol followed by drying the upper n-butanol layer in small glass tubes under vacuum to obtain the major cs oxidant with a purity of 98-99% and yield of 18-22 μg per cigarette, and
 - (f) purifying the above said cs oxidant as obtained in step e by dissolving it in a mobile solvent comprising a mixture of methylene chloride and methanol in a ratio of 90:10(v/v) and injecting it in a HPLC instrument with a normal phase 25 cm silica column using a uv detector at 294 nm at a flow rate of 0.5 ml/min, at a temperature of about 25°C , at a pressure of about 29 kgf/cm² and collecting the effluent which appears as a single peak at a retention time of 8.808 min with a purity of 100 % and yield of 8.4% of the total cs oxidant present in the parent cs solution.

Claim 6 (previously presented): A process as claimed in claim 1, wherein p-benzosemiquinone present in cs solution is quantitatively assayed by HPLC with a UV detector using a 25 cm reverse phase ODS column and using a mixture of water and methanol (95: 5 v/v) as a mobile phase, at a wave length of 288nm, flow rate of 0.8 ml/min, at a temperature of about 25°C and at a pressure of about 147 Kg/cm² and having a retention time of 13.46 min.

Claim 7 (original): A process as claimed in claim 1, wherein the said p-benzosemiquinone is responsible for the major cause of oxidative damage of proteins isolated from the whole cs solution.

Claim 8 (original): A process as claimed in claim 1, wherein p-benzosemiquinone, the cs oxidant is responsible for the oxidative damage of DNA.

Claim 9 (original): A process as claimed in claim 1, wherein the damage of proteins caused

by p-benzosemiquinone present in cs solution is quantitatively determined by measuring protein carbonyl formation by reacting the protein with p-benzosemiquinone obtained from the cs solution, followed by reaction with 2,4 dinitrophenyl hydrazine (DNPH) and finally measuring the absorbance at a wave length of 390nm.

Claim 10 (original): A process as claimed in claim 1, wherein the damage of proteins caused by p-benzosemiquinone present in cs solution is quantitatively determined by measuring oxidative degradation of guinea pig tissue microsomal proteins by reacting the said protein with p-benzosemiquinone present in cs solution followed by SDS-PAGE and densitometric scanning.

Claim 11 (original): A process as claimed in claim 10, wherein the protein used for the assay of oxidative damages of protein is selected from the group consisting of BSA and guinea pig lung microsomal proteins.

Claim 12 (original): A process as claimed in claim 10, wherein the BSA oxidation produced by the whole cs solution is effected by the p-benzosemiquinone present in the cs solution.

Claim 13 (original): A process as claimed in claim 12, wherein the BSA oxidation produced by the cs oxidant as evidenced by nmoles of carbonyl formed per mg BSA is 9.56 ± 0.14 in comparison to 7.53 ± 0.34 produced by the whole cs solution.

Claim 14 (original): A process as claimed in claim 12, wherein the BSA oxidation produced by the cs oxidant as evidenced by nmoles of carbonyl formed per mg BSA is 9.56 ± 0.14 in comparison to 8.16 ± 0.24 produces by the aqueous extract of cigarette smoke.

Claim 15 (original): A process as claimed in claim 12, wherein the BSA oxidation produced by the cs oxidant as evidenced by nmoles of carbonyl formed per mg BSA is 9.56 ± 0.14 in comparison to 9.23 ± 0.14 produces by the TLC purified aqueous extract of cigarette smoke.

Claim 16 (original): A process as claimed in claim 11, wherein the oxidative degradation of guinea pig tissue microsomal proteins produced by the p-benzosemiquinone solution as

evidenced by SDS-PAGE is comparable to that produced by the whole cs solution.

Claim 17 (original): A process as claimed in claim 1, wherein the said method is used for quantitative determination of cs oxidant p-benzosemiquinone in cigarettes based on the tar content of the particular commercial brand of the cigarette.

Claim 18 (original): A process as claimed in claim 1, wherein the said method is used for quantitative determination of cs oxidant p-benzosemiquinone in cigarettes based on toxicity level of the particular commercial brand of the cigarette.

Claim 19 (original): A process as claimed in claim 1, wherein the amount p-benzosemiquinone isolated from smoke of different commercial brands of burning cigarettes is used to determine the toxicity index of a particular brand of cigarette based on the quantity of p-benzosemiquinone present.

Claim 20 (withdrawn): A method for the prevention of cigarette smoke induced protein oxidation in vitro, said method comprises inhibiting the BSA oxidation by using a chemical compound or agent selected from the group consisting of ascorbic acid, sodium dithionite, tartaric acid, citric acid, oxalic acid, succinic acid, histidine, lysine, thiourea, glutathione, black tea extract, green tea extract, catechin, epigallocatechin and epicatechin.

Claim 21 (withdrawn): A method as claimed in claim 20 wherein ascorbic acid inhibits BSA oxidation up to 76% at a concentration of about 100 μ M.

Claim 22 (withdrawn): A method as claimed in claim 20 wherein Sodium dithionite inhibits BSA oxidation up to 97% at a concentration of about 2 mM.

Claim 23 (withdrawn): A method as claimed in claim 20 wherein tartaric acid inhibits BSA oxidation up to 75% at a concentration ranging between 500 μ M to 1 mM.

Claim 24 (withdrawn): A method as claimed in claim 20 wherein citric acid inhibits BSA

oxidation up to 75% at a concentration ranging between 500 μ M to 1 mM.

Claim 25 (withdrawn): A method as claimed in claim 20 wherein oxalic acid inhibits BSA oxidation up to 53% at a concentration of about 500 μ M.

Claim 26 (withdrawn): A method as claimed in claim 20 wherein succinic acid inhibits BSA oxidation up to 60% at a concentration of about 1mM.

Claim 27 (withdrawn): A method as claimed in claim 20 wherein histidine acid inhibits BSA oxidation up to 67% at a concentration of about 1mM.

Claim 28 (withdrawn): A method as claimed in claim 20 wherein black tea extract inhibits BSA oxidation up to 50% at a concentration of about 2.5mg.

Claim 29 (withdrawn): A method as claimed in claim 20 wherein catechin inhibits BSA oxidation up to 54% at a concentration of about 750 μ g.

Claim 30 (withdrawn): A method as claimed in claim 20 wherein epigallocatechin inhibits BSA oxidation up to 95% at a concentration of about 140 μ g.

Claim 31 (withdrawn): A method as claimed in claim 20 wherein epicatechin inhibits BSA oxidation up to 50% at a concentration of about 50 μ g.

Claim 32 (withdrawn): A method as claimed in claim 20 wherein green tea extract inhibits BSA oxidation up to 50% at a concentration of about 2.5mg.

Claim 33 (withdrawn): A method as claimed in claim 20 wherein lysine inhibits BSA oxidation up to 35% at a concentration of about 1mM.

Claim 34 (withdrawn): A method as claimed in claim 20 wherein thiourea inhibits BSA oxidation up to 52% at a concentration of about 10mM.

Claim 35 (withdrawn): A method as claimed in claim 20 wherein glutathione inhibits BSA oxidation up to 37% at a concentration of about 1mM.

Claim 36 (canceled)

Claim 37 (canceled)

Claim 38 (withdrawn): A method for quantitative estimation of an harmful oxidant, p-benzosemiquinone, the said method is helpful in formulating the quantity and nature of smoking material to be used in cigarette, cigar, cigarette pipes and any other convention smoking devices.

Claim 39 (withdrawn): A process as claimed in claim 2, wherein said isolated pure cigarette smoke (cs) oxidant has the following properties:

- (a) when crystallized from acetone solution appears as small needle shaped faint yellow coloured crystals having pungent smell, similar to that of rancid butterfat,
- (b) UV absorption maxima in methanol solution are at 293.4 nm and 223.0 nm and in aqueous solution are in 288nm and 221nm, respectively,
- (c) on excitation at 293 nm in methanol solution the observed emission maxima are at 329.6 nm and 651.4 nm and on excitation at 224 nm, the observed emission maxima are at 329.6 nm and 652.6 nm, respectively,
- (d) when excitation scanning is monitored keeping the emission at 330 nm, the observed excitation maxima are at 228.2 nm and 293.8 nm and when the emission is kept at 651 nm and excitation scanning is monitored, the observed excitation maxima are at 229.2 nm and 294.8 nm, respectively,
- (e) highly soluble in methanol, ethanol, acetone, n-butanol, fairly soluble in water, sparingly soluble in methylene chloride, di-ethyl ether, chloroform and insoluble in benzene and petroleum ether,
- (f) the compound loses its oxidizing potency in acidic pH ranging between 4 to 5 and on keeping the solution at alkaline pH ranging between 9 to 10, the compound gradually turns brown, at pH 10 and above there is instantaneous darkening with loss

- of both activity and aromaticity as evidenced by UV spectroscopy,
- (g) the half-life of the oxidant, when stored in the solid state at a temperature ranging between 25 °C to 30 °C under darkness is about 48 hours as determined by its oxidative potency, but in solution of 50 mM potassium phosphate buffer, pH 7.4 at 25°C to 30°C the half life is about 1hour 30 min,
 - (h) reduces ferricytochrome c and ferric chloride,
 - (i) oxidizes ascorbic acid , proteins and DNA, and
 - (j) the melting point is 162°C.

Claim 40 (withdrawn): A process as claimed in claim 2, wherein p-benzosemiquinone present in cs solution is quantitatively assayed by HPLC with a UV detector using a 25 cm reverse phase ODS column and using a mixture of water and methanol (95: 5 v/v) as a mobile phase, at a wave length of 288nm, flow rate of 0.8 ml/min, at a temperature of about 25°C and at a pressure of about 147 Kgf/cm² and having a retention time of 13.46 min.

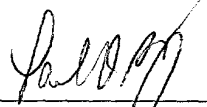
Claim 41 (previously presented): A process as claimed in claim 4, wherein p-benzosemiquinone present in cs solution is quantitatively assayed by HPLC with a UV detector using a 25 cm reverse phase ODS column and using a mixture of water and methanol (95: 5 v/v) as a mobile phase, at a wave length of 288nm, flow rate of 0.8 ml/min, at a temperature of about 25°C and at a pressure of about 147 Kgf/cm² and having a retention time of 13.46 min.

REMARKS/ARGUMENTS

Claims 1 through 35 and 38 through 41 are pending in this application. Claims 2, 5, 20 through 35, and 38 through 40 have been withdrawn.

The Office Action asserts that an election of a single invention is required, as defined by Species I (claims 1, 3 to 4, 6 to 19, and 41), drawn to a process for the isolation of / quantitative determination of p-benzosemiquinone of Formula 1 - involving collecting tart by lighting cigarettes in a glass flask dipped in a mixture of ice and sale, Species II (claims 2, 5, and 39 to 40), drawn to a process for the isolation of / quantitative determination of p-benzosemiquinone of Formula 1 - involving passing cigarette smoke from cigarettes into potassium buffer, or Species III (claims 20 to 35), drawn to a method for the prevention of cigarette smoke induced protein oxidation - involving inhibiting BSA oxidation by using a particular chemical compound or agent. Applicant respectfully traverses because each of the groups as set forth in the Office Action has the common invention set forth in independent claims 1, 2, 4, 5, 20, and 38. Notwithstanding the foregoing, to comply fully with the election requirement, applicants elect with traverse to prosecute species group I, which includes claims 1, 3 to 4, 6 to 19, and 41. Consideration and allowance of the application is respectfully requested.

Sincerely,



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